CCV. THE HYDROLYSIS OF CONCENTRATED SUGAR SOLUTIONS BY INVERTASE.

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Most of the extensive literature dealing with the kinetics of invertase action refers to the hydrolysis of dilute sucrose solutions. The commercial preparation of invert sugar from sucrose by hydrolysis in the presence of invertase necessitates the use of concentrated sugar syrups, and the object of the experiments reported was to study the relation between the rate of inversion and the sugar concentration.

Nelson and Schubert [1928] found that the velocity of hydrolysis by invertase decreased when the substrate concentration was greater than 10 %, the relation between sucrose concentration and velocity being approximately linear between 10 % and 70 % sucrose. This decrease in velocity was shown to be due to the falling off in water content.

Colin and Chaudun [1922] determined the relative viscosity of solutions containing from 10 to 60 g./100 cc. of sucrose and showed that the amount of sugar hydrolysed in a given time by a fixed amount of invertase decreased as the viscosity of the solution increased, the relation between fluidity and amount of hydrolysis being nearly linear. Since the viscosity of concentrated sucrose solutions is an exponential and not a linear function of the sugar content, it appears that the results of Colin and Chaudun are not in agreement with those of Nelson and Schubert.

The experiments of Colin and Chaudun were carried out at 23°, a temperature considerably lower than that of the critical inactivation of invertase. The optimum temperature for hydrolysis by invertase is generally taken to be 50–55° [*cf.* Oppenheimer and Kuhn, 1927; Waksman and Davison, 1926], but this value is not characteristic of the enzyme, being dependent on the time of the reaction. In view of the high negative temperature coefficient of viscosity, and the fact that an enzyme in the presence of its substrate is more resistant to high temperatures, it appeared probable that the apparent optimum temperature for the hydrolysis of concentrated sugar solutions by invertase would be higher than that for dilute substrate concentrations. The data obtained in the following experiments show that this is the case.

Experimental.

The invertase used was a very active preparation obtained from autolysed yeast. Its activity was tested from time to time and was found to remain constant during the period covered by the investigation. The stock enzyme solution was diluted immediately before use and the amount added to the substrate adjusted so that in all cases 100 cc. of sugar solution contained 0·4 cc. of invertase preparation. The stock enzyme solution was buffered with phosphoric acid to the extent that, when added in the above amount to sugar solutions, the resulting p_H was 4·4–4·6. No alteration in p_H was observed during the course of the hydrolysis.

To stop the hydrolysis 10 cc. of the reaction mixture were pipetted into a 100 cc. flask containing 80 cc. 0·25 % NaOH, and the solution was diluted with distilled water to 100 cc. at 15°. The mean delivery time of the pipette was taken as the time of observation. In view of the viscosity of the concentrated sugar solutions, the standard drain of the pipette was increased from 15 to 30 seconds, during which time the bulk of the sample was mixed with the alkali. The rotation of the sample was observed 15 to 30 minutes later. Readings were taken in a 400 mm. jacketed-tube round which water from a thermostat at 20° was circulated.

The amount of hydrolysis was calculated from the formula

$$\% \text{ hydrolysis} = \frac{R-d}{R-L}\,100,$$

where d is the observed rotation,
R is the initial rotation,
L is the rotation for complete inversion.

The initial rotation was determined for each concentration and temperature by adding to the sugar solutions the standard amount of invertase, the activity of which had been destroyed by diluting with NaOH. The final rotation was calculated from the relation

$$L = 0{\cdot}317\,R \text{ at } 20° \text{ [Hudson, 1910].}$$

The rotation of the invertase preparation was found to be negligible at the concentrations used.

The reaction velocity was calculated from the formula

$$k = \frac{1}{t}\log_{10}\frac{a}{a-x}.$$

In the experiments dealt with in Tables I–IV the amount of enzyme was constant, but the weight of substrate varied with the concentration. In order to calculate the activity of the enzyme in terms of the absolute weight of sugar hydrolysed in unit time, use was made of the expression:

$$\text{Invertase activity} = 10k \times \text{g. substrate.}$$

Table I. *The hydrolysis of solutions containing 70 g. sucrose/100 cc.*

T.	t (minutes)	% hydrolysis	$k \times 10^5$	
45°	56	27·6	250	
	88	39·2	247	
	143	57·0	255	
	163	60·1	246	Average 250
50°	60	36·8	332	
	87	49·2	338	
	137	65·9	341	
	178	75·0	338	Average 337
57°	44	39·2	491	
	73	56·2	491	
	112	72·2	496	
	144	79·3	496	
	204	89·4	(478)	Average 493
62·2°	19·5	24·5	626	
	41·5	45·8	643	
	83	69·3	618	
	114	79·8	610	Average 624
65°	15	20·84	(677)	
	40	48·4	719	
	75	70·8	714	
	115	84·1	695	Average 709
68·8°	15	25·2	840	
	41·5	54·9	833	
	76·5	76·1	813	
	120	88·4	(780)	Average 829
72°	17·5	31·4	935	
	41·5	58·0	908	
	81	78·7	(830)	
	119	87·9	(771)	Average 921
75°	26·5	41·4	876	
	50	57·4	743	
	83	69·3	618	
	128	78·8	526	

Table II. *Hydrolysis of solutions containing 55 g. sucrose/100 cc.*

T.	t (minutes)	% hydrolysis	$k \times 10^5$	
38°	54·7	35·2	344	
	91	53·5	365	
	128	66·4	370	
	163·7	75·6	374	
	225	86·0	379	Average 366
45°	25·2	25·1	(495)	
	60	51·7	527	
	88	66·5	540	
	117	76·7	541	
	165·5	86·5	526	Average 533
57°	30·7	47·0	898	
	56·3	70·2	933	
	80	81·7	922	
	107·5	89·2	898	Average 913
65°	20·5	44·3	1240	
	40	68·6	1260	
	71	87·8	1280	
	100	93·6	1190	Average 1240

Table III. *Hydrolysis of solution containing 40 g. sucrose/100 cc.*

T.	*t* (minutes)	% hydrolysis	$k \times 10^5$	
57°	18	49·5	1650	
	26·3	63·4	1660	
	25·7	75·0	1690	
	51·75	86·6	1680	Average 1680

Table IV. *Hydrolysis of solution containing 70 g. sucrose/100 g. solution (94·5 g./100 cc.).*

Digestion mixture 70 g. sucrose, 29·6 cc. water, 0·4 cc. invertase solution.

T.	*t* (minutes)	% hydrolysis	$k \times 10^5$	
57°	42	12·8	142	
	90·5	25·5	141	
	169	42·1	140·5	
	296	58·5	(130)	Average 141
65°	20	10·1	231	
	54	25·6	238	
	105	43·4	235	
	175	57·2	(211)	
	277	70·4	(191)	Average 235
70°	28·5	18·9	319	
	65·5	36·9	306	
	117·5	53·4	282	
	203	69·1	252	
	308	79·2	222	

The bracketed values for *k* were not used in estimating the mean value.

Table V.

T.	Concentration g./100 cc.	$10k$	$10k \times$ g. substrate
57°	40	0·167	6·68
	55	0·0913	5·02
	70	0·0493	3·45
	94·5	0·0141	0·99
65°	55	0·124	6·82
	70	0·0709	4·96
	94·5	0·0235	1·64

Fig. 1 shows that the relation between invertase activity and substrate concentration is linear.

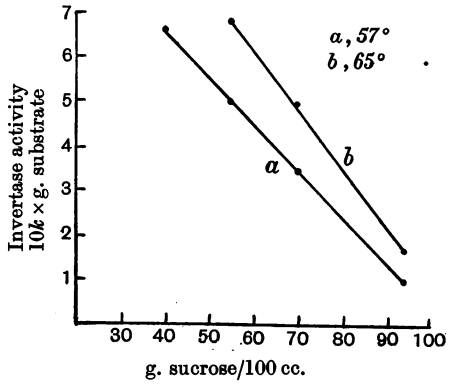

Fig. 1. Relation between invertase activity and substrate concentration.

The temperature coefficient.

From the results given in Table I it appears that for a digestion period of a few hours the apparent optimum temperature for the hydrolysis of 70 % sucrose in the presence of invertase is about 70°.

Experiments on the hydrolysis of 10 % sucrose by invertase at the same p_H showed that at 65° the value for k decreased very rapidly, while at 68° the hydrolysis ceased after 20 minutes owing to the inactivation of the enzyme.

The temperature coefficient of the reaction is also greater for concentrated, than that found by Nelson and Bloomfield [1924] for dilute, solutions. Calculation of the values of the critical increment in calories per gram-molecule gave the following results:

Table VI.

$$E = R\frac{T_1 - T_2}{T_2 - T_1}\log_e \frac{k_2}{k_1}.$$

Conc. of sugar	$T°$ (abs.)	$k \times 10^5$	Temp. range ° C.	Mean temp. $t°$ C.	E (found)	E (calc.)
70 %	318	250	45 –50	47·5	12,300	12,500
	323	337	45 –57	51	11,900	11,900
	330	493	50 –57	53·5	11,600	11,500
	335·2	624	57 –65	61	10,100	10,200
	338	709	57 –68·8	62·9	9,900	9,900
	341·8	829	62·2–72	67·1	9,200	9,200
	345	921	65·2–72	68·6	8,900	9,000
55 %	311	366	38 –45	41·5	10,600	10,600
	318	533	45 –57	51	9,400	9,600
	330	913	57 –65	61	8,500	8,500
	338	1240	—	—	—	—
94·5 %	330	141	57 –65	61	14,200	—
	338	235	—	—	—	—

The experimental values for E are plotted against the mean temperature in Fig. 2 and are in agreement with the equations

$$E = 20{,}500 - 168t \text{ for } 70\ \%\ \text{sucrose},$$
$$E = 15{,}100 - 108t \text{ for } 55\ \%\ \text{,,}\ .$$

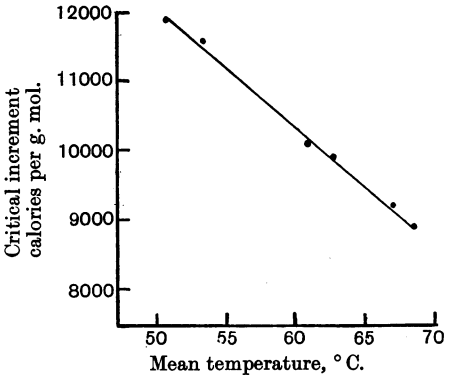

Fig. 2. Variation of the critical increment with temperature. Sucrose concentration 70 g./100 cc.

Nelson and Bloomfield [1924] combined the data in the literature for the hydrolysis of dilute sugar solution by invertase and found that the observed values could be calculated from the equation

$$E = 12{,}300 - 117t.$$

In order to ensure that the divergence between the values for E for the different substrate concentrations was not due to the enzyme preparation, the rate of hydrolysis of 10 % sucrose by invertase at p_H 4·6 was determined at 38° and 56·5°. The values of k for the unimolecular reaction were found to increase regularly as the hydrolysis proceeded and a different method was used to calculate the relative rates of hydrolysis at the two temperatures. The time t_z required by the reaction mixture to reach zero rotation (75·9 % hydrolysis) was found to be 61 and 32 minutes at 38° and 56·5° respectively. Using the reciprocals of these values to denote the relative velocities, the value of E was calculated to be 7100: the corresponding figure calculated from the equation of Nelson and Bloomfield for a mean temperature of 47·5° was 6800.

Viscosity and rate of hydrolysis.

In order to test the statement of Colin and Chaudun that the amount of hydrolysis by invertase of concentrated sucrose solutions is proportional to the fluidity of the solution, the viscosities of the solutions used were calculated. For this purpose Orth's equation [Browne, 1912] relating the viscosity of concentrated sugar solutions to the temperature and concentration, was used:

$$\log_e(\log_e \eta) = \log_e(\log_e A) + x \log_e B + t \log_e C,$$

where η is the viscosity,

x is the concentration in g./100 g. solution,

t is the centigrade temperature, and

A, B and C are constants.

Applying viscosity data from Browne [1912] and from Landolt-Börnstein [1923] the equation was simplified to the following:

$$\log_e(\log_e \eta) = 3{\cdot}619 + 0{\cdot}07798x - 0{\cdot}02260t.$$

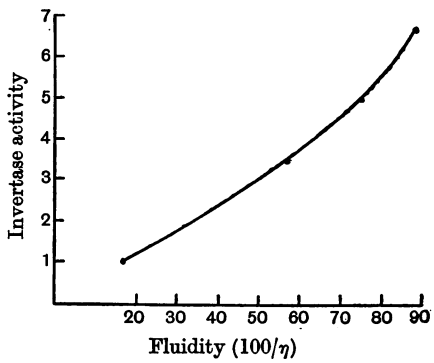

Fig. 3. Relation between the fluidity of sugar solutions and invertase activity. Temperature 57°.

In Fig. 3 the fluidity ($100/\eta$) of sucrose solutions at 57° is plotted against the invertase activity (Table VI) for the corresponding concentration. The linear relationship found by Colin and Chaudun is not observed.

Discussion.

According to Euler and Laurin [1919] the critical inactivation temperature for invertase is 59°. The observations described above lead to the conclusion that invertase resists exposure to much higher temperatures when in the presence of a large excess of substrate. The increased rate of hydrolysis observed at temperatures above that of critical inactivation is not merely an increase in the initial velocity, since the observations covered a large portion of the range of inversion. In this connection it should be noted that the course of the hydrolysis by invertase of 70 % sucrose follows that of a unimolecular reaction much more closely than does the hydrolysis of 10 % sucrose, although deviations from the mass law might be expected with the more concentrated solutions.

The larger values of the temperature coefficient of the hydrolysis of concentrated, compared with that for dilute, solutions cannot be ascribed to the increased rate of hydrolysis due to decrease in viscosity with rise in temperature, since this would not account for the greater resistance to temperature of the enzyme in presence of 70 % sugar. A concentration of 10 % sucrose is greater than that required to produce the maximum initial velocity of hydrolysis by invertase [*cf.* Kuhn, 1923], and presumably the enzyme is already saturated with substrate at this concentration. By applying the conception of Willstätter that an enzyme consists of an active substance stabilised by the presence of a colloidal carrier, the resistance of the enzyme-complex to temperature in the presence of sugar syrup may be explained as due to the increased stability of the carrier in this medium.

In Figs. 1 and 3 it is shown that the decrease in the rate of hydrolysis with increasing sucrose concentration is more closely related to the decrease in water content than to the increase in viscosity. This is in agreement with the results of Ingersoll [1926] who found that the initial velocity of sucrose hydrolysis by invertase at 25° did not vary with the increase in viscosity due to increase in sugar concentration in the manner described by Colin and Chaudun. Ingersoll also showed that the initial velocity was not decreased by the addition of gelatin in amounts sufficient to produce a considerable increase in viscosity. It may be concluded, therefore, that the viscosity of the medium is not the factor responsible for the observed variations in the catalytic activity of invertase.

The values calculated for the critical increment are subject to a possible correction for the variation of the optimum p_H with temperature and substrate concentration. Nelson and Bloomfield, however, have shown that the small shift in optimum p_H with temperature is towards the alkaline side. This means that the actual estimations were carried out slightly on the acid side of the

optimum. Since Nelson and Bloomfield have shown that the value of the critical increment is smaller at acidities greater than the optimum, it follows that any correction would increase the value of E for the concentrated solutions, and so increase the difference between the values for the dilute and concentrated substrate.

SUMMARY.

1. The rate of hydrolysis by invertase of solutions containing 40 % or more of sucrose decreases as the substrate concentration increases, the relation between substrate concentration and invertase activity being linear.

2. The optimum temperature for the hydrolysis of 70 % sucrose in the presence of invertase is 65–70°.

3. The relation between the critical increment in calories per gram-molecule and the centigrade temperature for the hydrolysis of 70 % sucrose by invertase can be expressed by the relation

$$E = 20{,}500 - 168t.$$

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